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Analysis of Invasive *Haemophilus influenzae* Infections after Extensive Vaccination against *H. influenzae* Type b

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Little clinical and microbiological information is available about invasive *Haemophilus influenzae* infection after widespread vaccination against *H. influenzae* type b (Hib). We conducted an active community surveillance study on invasive *H. influenzae* during a 2-year period in a community of more than 5 million people after vaccination against Hib in children was introduced. The median incidence was 16.3 cases/100,000 persons per year in children less than 1-year-old and 4.41 cases/100,000 persons in children less than <5 years old. The highest incidence in adults was observed in patients greater than 70 years old. Clinical diagnoses included bacteremia, pneumonia, and meningitis. Of the *H. influenzae*-infected patients, 74.3% had underlying predisposing conditions, including impaired immunity and respiratory diseases. A total of 73.6% of the isolates were nontypeable and 16.5, 6.6, and 3.3% were types b, f, and e, respectively. Infections due to capsulated strains b, e, and f were evenly distributed between children and adults. Ampicillin and cotrimoxazole resistance occurred at frequencies of 24.2 and 48.4%, respectively. Antibiotic resistance was more prevalent in capsulated than in noncapsulated *H. influenzae*. Invasive isolates were highly resistant to antibiotics that were used infrequently in the community. Nontypeable *H. influenzae* were genetically much more heterogeneous than capsulated strains. Capsule-deficient mutants (b^-) were not detected. Plasmid carriage was linked to antibiotic resistance and capsulated strains. Over the study period, the incidence of invasive *H. influenzae* infections, either encapsulated or not, did not increase. In the post-Hib vaccination era, most invasive infections were due to noncapsulated strains and occurred in the extreme ages of life in patients with predisposing conditions.

Haemophilus influenzae can be typed according to capsular antigen composition into six capsular serotypes (a to f) and into nontypeable strains. *H. influenzae* type b (Hib) is the most invasive type and is recognized to be an important cause of pneumonia and meningitis (10). It used to be one of the most prevalent bacterial pathogens causing meningoencephalitis in children under 5 years of age; however, invasive diseases caused by Hib can be prevented by immunization with a polysaccharide-protein conjugate vaccine. With the widespread use of effective conjugate vaccines, infections by Hib and the prevalence of carriers have decreased substantially (6, 30), although vaccine failures have also been detected (8, 28).

Vaccination campaigns generate a novel epidemiological environment. In theory, the decline in the rate of Hib infections could encourage the emergence of diseases caused by other *H. influenzae* serotypes (7, 35). An increase in invasive infections caused by *H. influenzae* type f has been described in United States (35). In our experience, infections due to other *H. influenzae* serotypes occur mainly in adult patients with underlying diseases (9).

In the Madrid area, a widespread program of vaccination against *H. influenzae* type b has been in place since 1998, although conjugate vaccines had been available for private use almost 2 years before. Conjugate Hib vaccine was given at 2, 4, 6, and 18 months of age. Population coverage was >95%. The incidence of invasive infections due to *H. influenzae* in the Madrid area was 20 cases/100,000 inhabitants <5 years of age in 1994, although detailed serotype distribution information was not available (15).

Some time ago, Spain recorded high rates of antimicrobial resistance in *H. influenzae* (11, 12). Spain is also a country with high antibiotic consumption in comparison with other European countries (13).

Little information is available about the epidemiology, microbiology, and molecular epidemiology of invasive *H. influenzae* after the widespread vaccination campaigns with Hib conjugate vaccines. Accordingly, we sought to evaluate here the incidence of invasive *H. influenzae* in the population, to study the demographic features of the patients infected, to learn about their clinical patterns and possible predisposing underlying conditions, to determine the molecular epidemiology of the isolates, and to study their antimicrobial susceptibility patterns in connection with antimicrobial consumption in the same population.

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MATERIALS AND METHODS

Microbiological identification. Invasive strains were sent to a central laboratory (Majadahonda, Madrid), where full microbiological identification, susceptibility testing, and molecular epidemiology studies were carried out by the classical slide agglutination test with type-specific antiserum (Difco Laboratories,

Detroit, Mich.) and the molecular PCR method (10), which was considered to be the reference test.

Population and clinical study. From January 1999 to December 2000, all patients with *H. influenzae* invasive infection diagnosed in clinical laboratories in the Madrid area were prospectively studied. Invasive infection was defined as "infection with an *H. influenzae* isolate from a normally sterile site." All available microbiological laboratories and hospitals of the Madrid area (Autonomous Community of Madrid [ACM]), which serve a population of 5,022,289 (1996 census data), were contacted at the beginning of the study and regularly afterward; all of them were asked to send all of their invasive *H. influenzae* isolates to the reference laboratory. According to official census data, the structure of the Madrid population was very similar to the rest of Spain and other countries of the European Union. A medical epidemiologist examined the clinical records of the patients. An individual clinical protocol that included patient's identity data, clinical pictures, diagnosis, and a description of underlying conditions and outcome was filled out for each patient. General incidence, expressed as cases per 100,000 people per year, and specific incidence by age group were calculated. Incidence in children <5 years of age was compared to data obtained in 1994, before conjugate vaccines were available in the ACM (15).

Molecular studies. All clinical strains were examined by pulsed-field gel electrophoresis after digestion of bacterial DNA with *Sma*I (MBI Fermentas, Vilnius, Lithuania) as described previously (9). DNA fragments were analyzed by the UPGMA (unweighted pair-group method with arithmetic averages) clustering method and Dice coefficient, which are available as part of the Molecular Analyst program (Bio-Rad, Madrid, Spain).

Screening of large conjugative plasmid carriage was carried out by PCR, as described by Leaves et al. (22). Screening of capsule-deficient type b strains (b^- phenotype) was determined by PCR as described earlier (23).

Antimicrobial susceptibility testing. Testing for antimicrobial susceptibility to 14 antimicrobial agents was performed according to National Committee for Clinical Laboratory Standards guidelines (29) by using a semiautomated microdilution method (Wider; Fco. Soria Melguizo S.A., Madrid, Spain). *H. influenzae* ATCC 49247 and *H. influenzae* ATCC 49766 were used as quality control strains as recommended elsewhere (15).

β-Lactamase production was determined by the chromogenic cephalosporin test with nitrocephin as substrate. Chloramphenicol acetyltransferase was measured as described previously (4).

Antibiotic consumption. Due to the published high rates of antibiotic resistance in *H. influenzae* in Spain (11, 12), we studied the evolution of antibiotic consumption in the ACM. The Spanish Ministry of Health and Consumer Affairs maintains a drug database with a packet-by-packet record of all retail pharmacy sales of all medicines acquired with National Health System prescriptions (21, 31). This database was used to gather information on sales in the ACM over the period from 1996 to 2000. The information was tabulated, and the number of units sold was converted into defined daily doses (DDDs) of active drug ingredients according to World Health Organization (WHO) methodology (37). The number of DDDs per 1,000 inhabitants per day (DDD/1,000 inhabitants/day) for each of the active drug ingredients was then calculated.

Statistical analyses. Data were managed and statistics calculated by using the SPSS program (SPSS, Inc., Chicago, Ill.). Categorical variables were compared by two-tailed Fisher exact test. Association was determined by calculation of the odds ratio and its 95% confidence interval. The null hypothesis was rejected for P values of <0.05.

RESULTS

Microbiological identification. A total of 113 *H. influenzae* isolates were obtained; 91 (80.5%) of these were from blood culture, 12 (10.6%) were from cerebrospinal fluid (CSF), 5 (4.4%) were from pleural fluid, 3 (2.6%) were from abdominal fluid, and 2 (1.8%) were from biopsy. In 91 cases (80.5%), the clinical isolates were available for microbiological studies; of these, 67 strains (73.6%) were nontypeable, whereas 15 (16.5%), 6 (6.6%), and 3 (3.3%) strains belonged to serogroups b, f, and e, respectively. Of the 24 capsulated strains isolated during the study period, 12 were obtained in 1999 (7 type b, 4 type f, and 1 type e) and 12 were obtained in 2000 (8 type b, 2 type f, and 2 type e). The proportion of *H. influenzae* types e and f was 9.8% (in 1999) and 10% (in 2000) ($P = 1.0$), whereas the proportion of noncapsulated isolates was 76.4% (in 1999) and 70% (in 2000) ($P = 0.6$).

(i) Population study. A total of 113 patients had *H. influenzae* invasive infections in the ACM. Males accounted for 75 (66.4%) cases and females for 38 (33.6%) ($P < 0.001$). The median incidence in the total population was 1.0 cases/100,000 persons per year, without differences between the 2 years studied. The median incidence of noncapsulated and capsulated *H. influenzae* strains was 0.7 and 0.3 cases/100,000 persons per year, respectively. No differences were observed between the 2 years studied.

In four (3.3%) cases, the patient's age was missing; of the remaining 109, 81 (74.3%) were adults (>14 years) and 28 (25.7%) were children (≤14 years), 22 (19.5%) patients were <5 years old, and 14 (12.4%) were <1 year old.

The incidence of *H. influenzae* invasive infections was higher in patients ≤14 years old than in those >14 years ($P < 0.001$) (Fig. 1). The highest incidence was detected in children <1 year of age, with 16.3 cases/100,000 inhabitants (Fig. 1). In adults, the highest incidence (2.6/100,000 inhabitants) was in patients >70 years old (Fig. 1).

Of the noncapsulated *H. influenzae* strains, 55 (80.3%) caused infections in patients >14 years old, whereas only 14 of them were isolated from patients who were ≤14 years old ($P = 0.005$). No significant differences were detected between the two age groups in the number of infections caused by capsulated strains. In children <5 years old, the prevalence of invasive infections due to typeable and nontypeable *H. influenzae* strains did not increase over the 2-year study period; in this age group, eight capsulated strains (four serotype b in 1999 and three serotype b and one serotype e in 2000) were isolated from CSF or blood; all serotype b cases were found in unvaccinated or partially vaccinated children.

In comparison with data obtained before the implementation of conjugate Hib vaccines, the incidence of invasive *H. influenzae* decreased by 86% in children <5 years old (15).

(ii) Clinical study. *H. influenzae* caused bacteremia in 59 (52.2%) cases, pneumonia in 23 (20.4%) cases, and meningitis in 12 (10.6%) cases. In capsulated *H. influenzae* infections, meningitis and pneumonia were the second and third most frequent clinical diagnosis, after bacteremia. Of the pneumonia cases, 79% were caused by nontypeable *H. influenzae* strains and 21% were caused by capsulated strains ($P < 0.001$).

In all age groups, bacteremia was the most important clinical presentation. Pneumonia was more prevalent in adult patients (>14 years) than in children (≤14 years): 25.9% versus 7.1% ($P = 0.03$). In contrast, meningitis was more frequent in children than in adults, at 14.3% versus 9.8%, although this difference was not statistically significant; however, meningitis due to capsulated *H. influenzae* was more prevalent in children ($P = 0.03$).

Eighty-four patients (74.3%) had previous underlying predisposing conditions. The most prevalent underlying diseases were those that impaired immunity: tumor pathologies, organ transplantation, and primary impaired immunity in 36 (31.8%) cases, respiratory diseases in 26 (23%) cases (17 of whom [15%] had chronic obstructive pulmonary disease), intravenous drug use in 15 (13.3%) cases, human immunodeficiency virus infection in 10 (8.8%) cases, and premature birth and/or obstetric complications in 10 (8.8%) cases.

Of all of the 113 patients studied, 17 (15%) died. All of the

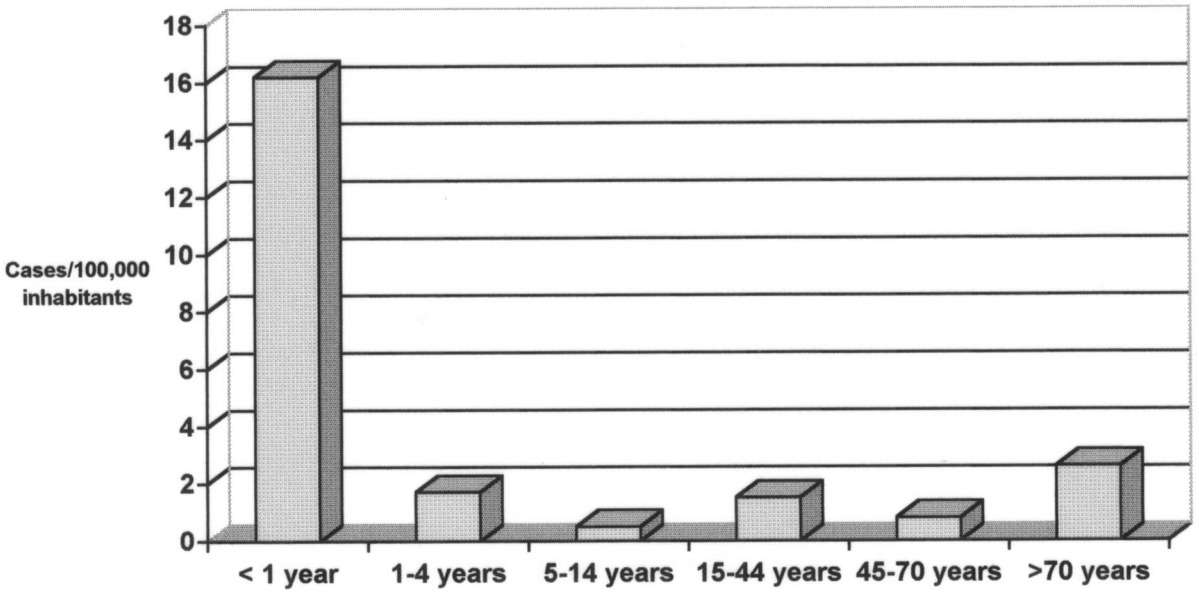

FIG. 1. Distribution of incidence (cases per 100,000 inhabitants) of *H. influenzae* invasive infections according to patient age.

patients who died were adults, and 55% of them were >65 years old.

Molecular studies. Cluster analyses of results of DNA fingerprinting of capsulated *H. influenzae* is shown in Fig. 2. *Sma*I did not digest the DNA of seven isolates, all of them nontypeable *H. influenzae*. Nontypeable *H. influenzae* isolates showed little genetic homology (data not shown); in contrast, capsulated strains were more homologous, since most serotype b strains had a genetic distance of ≤18% (Fig. 2).

Carriage of large conjugative plasmids was detected in 13 (11.5%) *H. influenzae* strains. Of the isolates resistant to ampicillin, tetracycline, and chloramphenicol, 59.1, 85.7, and 100%, respectively, carried large conjugative plasmids, whereas none of the fully antibiotic susceptible ones did (Table 1). Of these, all were excised, and seven were also chromosomally integrated. Of capsulated *H. influenzae*, 37.5% had positive plasmid detection in comparison with 6% in noncapsulated isolates ($P < 0.001$). There were no statistically significant

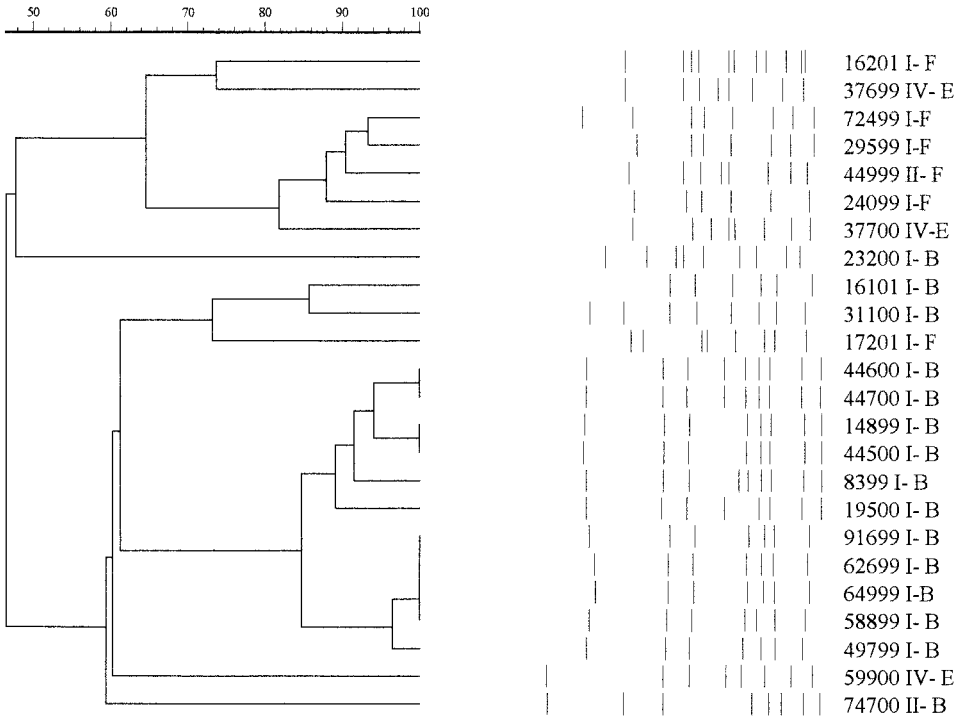

FIG. 2. Dendrogram tree illustrating the genetic relationship of 24 strains of capsulated *H. influenzae*, as determined by pulsed-field gel electrophoresis. The numbers at the right are strain numbers. The letters indicate biotypes (before hyphen) and serotypes (after hyphen).

TABLE 1. Frequency of antimicrobial resistance of invasive *H. influenzae* in relation to large conjugative plasmid carriage

Response to antibiotic	No. of strains (%)		P^a
	With plasmid (n = 13)	Without plasmid (n = 78)	
Susceptible	0	31 (39.7)	0.003
Nonsusceptible to:			
Ampicillin	13 (100)	9 (11.5)	<0.0001
Tetracycline	6 (46.2)	1 (1.3)	<0.0001
Cotrimoxazole	11 (84.6)	33 (42.3)	0.005
Clarithromycin	0	18 (23.1)	0.05
Chloramphenicol	6 (46.1)	0	<0.0001

[a] As determined by the Fisher exact test (two tailed).

differences in carriage of plasmids in pediatric and adult isolates.

Capsulate-deficient serotype b (b^- phenotype) *H. influenzae* isolates were not detected.

Susceptibility testing. MICs for the two control strains were always within recommended limits (29). A total of 24.2% of the isolates were resistant to ampicillin by β-lactamase production. Decreased susceptibility to cotrimoxazole, clarithromycin, tetracycline, chloramphenicol, and rifampin was detected in 48.4, 19.8, 7.7, 6.6 and 2.2% of strains, respectively. Resistance to amoxicillin-clavulanic acid, cefuroxime, cefotaxime, cefixime, cefepime, meropenem, ciprofloxacin, and levofloxacin was not observed.

The prevalence of susceptibility and the 50 and 90% MICs for *H. influenzae* isolates are presented in Table 2.

Ampicillin resistance was more prevalent in capsulated *H. influenzae* isolates (45.8%) than in noncapsulated ones (16.4%) ($P = 0.004$). Capsulated strains were also less susceptible to cotrimoxazole (83.3% versus 35.95%, $P < 0.001$) and chloramphenicol (20.8% versus 1.5%, $P < 0.004$) than noncapsulated strains. In contrast, decreased susceptibility to clarithromycin was higher in noncapsulated strains (23.9%) than in capsulated ones (8.3%), although this finding was without statistical significance.

Pediatric isolates were more resistant to ampicillin and cotrimoxazole than were adult isolates (44% versus 17.1% and 48% versus 28.6%, respectively), although only the difference with ampicillin was statistically significant ($P = 0.007$).

Ampicillin resistance was associated with decreased susceptibility to tetracycline, cotrimoxazole, and chloramphenicol ($P < 0.001$). However, decreased susceptibility to clarithromycin was more frequent in ampicillin-susceptible isolates (24.6%) than in ampicillin-resistant isolates (4.5%) ($P = 0.03$).

Isolates from blood and CSF had higher rates of resistance to ampicillin (27.5%) than those from other sterile sites (0%) ($P = 0.05$). In contrast, resistance to clarithromycin was less prevalent in isolates from blood and CSF than in those from other samples (17.7% versus 36.4%).

Multidrug resistance (nonsusceptibility to three or more antibiotics) was recorded for 10 (11%) of the 91 *H. influenzae* isolates. The most prevalent resistance phenotype was ampicillin-tetracycline-chloramphenicol, which was detected in three isolates, representing 33.3% of the multiresistant strains and 3.3% of strains overall.

TABLE 2. Susceptibility of 91 *H. influenzae* isolates from sterile sites in relation to noncapsulated and capsulated strains[a]

Antibiotic	Noncapsulated strains (n = 67)					Capsulated strains (n = 24)				
	MIC_{50}	MIC_{90}	Range	S (%)	I (%)	R (%)	MIC_{50}	MIC_{90}	Range	S (%)	I (%)	R (%)
Ampicillin	0.25	>4	0.12–>4	56 (83.6)	0	11 (16.4)	0.5	>4	0.25–>4	13 (54.2)	0	11 (45.8)
Amoxicillin-clavulanic acid	≤0.5	1	≤0.5–1	67 (100)	0	0	≤0.5	≤0.5	≤0.5–1	24 (100)	0	0
Cefuroxime	0.5	1	0.25–2	67 (100)	0	0	0.5	1	0.25–2	24 (100)	0	0
Cefotaxime	≤0.03	0.06	≤0.03–0.12	67 (100)	0	0	≤0.03	0.06	≤0.03–0.06	24 (100)	0	0
Cefixime	≤0.25	≤0.25	≤0.25	67 (100)	0	0	≤0.25	≤0.25	≤0.25	24 (100)	0	0
Cefepime	0.12	0.5	≤0.06–0.5	67 (100)	0	0	0.12	0.5	≤0.06–0.5	24 (100)	0	0
Meropenem	≤0.12	≤0.12	≤0.12–0.5	67 (100)	0	0	≤0.12	≤0.12	≤0.12	24 (100)	0	0
Clarithromycin	8	16	1–>16	51 (76.1)	13 (19.4)	3 (4.5)	4	8	2–16	22 (91.7)	2 (8.3)	0
Tetracycline	0.5	0.5	≤0.25–>4	64 (95.5)	1 (1.5)	2 (3)	0.5	>4	≤0.25–>4	20 (83.3)	3 (12.5)	1 (4.2)
Ciprofloxacin	≤0.06	≤0.06	≤0.06	67 (100)	0	0	≤0.06	≤0.06	≤0.06	24 (100)	0	0
Levofloxacin	≤0.25	≤0.25	≤0.25	67 (100)	0	0	≤0.25	≤0.25	≤0.25	24 (100)	0	0
Cotrimoxazole	≤0.5	>2	≤0.5–>2	43 (64.2)	6 (9)	18 (26.9)	>2	>2	≤0.5–>2	4 (16.7)	6 (25)	14 (58.3)
Chloramphenicol	≤2	≤2	≤2–>4	66 (98.5)	1 (1.5)	0	≤2	>4	≤2–>4	19 (79.2)	3 (12.5)	2 (8.3)
Rifampin	≤0.5	1	≤0.5–2	65 (97)	2 (3)	0	≤0.5	≤0.5	≤0.5–1	24 (100)	0	0

[a] MIC_{50}, MIC_{90}, and range values are expressed in micrograms per milliliter. S, susceptible; I, intermediate; R, resistant.

TABLE 3. Antimicrobial consumption in the ACM, 1996 to 2000

Code	Antibiotic	Consumption (DDD/1,000 inhabitants/day) in:				
		1996	1997	1998	1999	2000
J01A	Tetracycline	0.70	0.64	0.63	0.59	0.58
J01B	Amphenicol	0.01	0.01	0.00	0.00	0.00
J01C	β-Lactam anti-bacterials and penicillin	10.86	10.37	10.44	10.29	9.62
	Penicillin with extended-spectrum	6.35	5.96	5.69	5.48	5.24
	Amoxicillin-clavulanate	4.04	3.94	4.28	4.34	3.93
J01DA	Cephalosporin	1.60	1.39	1.35	1.25	1.09
J01EE	Trimethoprim-sulfa-methoxazole	0.71	0.66	0.56	0.47	0.43
J01F	Macrolide and lincosamide	2.41	2.26	2.46	2.30	2.14
J01M	Quinolone	1.53	1.45	1.43	1.50	1.55
Total		18.40	17.34	17.42	16.94	15.91

Antibiotic consumption. Antibiotic use in the ACM decreased from 18.40 DDD/1,000 inhabitants/day (in 1996) to 15.91 DDD/1,000 inhabitants/day (in 2000). The same trend was observed in almost all single antibiotic groups. β-Lactams (J01C group), principally penicillins, were the most widely used antibiotics. The consumption of tetracyclines (J01A group) and trimethoprim-sulfamethoxazole (J01EE group) was very low and decreased over time. Very little use of amphenicols (J01B group) was observed (Table 3).

DISCUSSION

The incidence of Hib invasive disease and oropharyngeal carriage in young children has drastically decreased wherever vaccination programs have been implemented (5, 14, 26). Little is known about the general epidemiology and clinical significance of invasive *H. influenzae* infection after the widespread vaccination with Hib conjugate vaccines. Falla et al. (19) found that 57% of nonserotypeable *H. influenzae* were capsulate deficient mutans (b^-) strains from serotype b vaccine recipients; in the present study, we did not confirm these results.

Worldwide reports of invasive *H. influenzae* isolates during the prevaccination era noted that >80% of the cases were caused by Hib (16, 20, 38). In a study carried out in England and Wales, Hib strains represented ca. 84% of all invasive isolates, whereas only 1% were of another capsular serotype (34). In a recent report from Brazil, Hib was by far the most common serotype (97.8%), followed in frequency by nontypeable strains (1.5%) and by serotype a (0.5%) (38). In comparison, epidemiology studies after vaccine implementations reveal a very different pattern (17, 34). In the present study, Hib invasive infections accounted for only 16.5% of invasive *H. influenzae* infections, results similar to the rates obtained in another Spanish study (17); both datasets were obtained after effective conjugate vaccination. The data from England showed that, since the introduction of routine immunization of infants with conjugate Hib vaccine, there has been a 16-fold reduction in the annual attack rate of invasive Hib disease recorded in children <5 years of age (34).

We noticed that 9 of 24 capsulate isolates were not serotype b; 6 of them were serotype f. In the United States the decrease in Hib infections has led to the report of increased incidence of *H. influenzae* type e and f (35, 36). The proportion of all invasive *H. influenzae* disease caused by serotype f rose from 1% in 1989 to 17% in 1994 in the United States (35). Previous Spanish results showed a 3% invasive *H. influenzae* type f infections (17). In comparison, in the present study we detected 6.6% of invasive *H. influenzae* infections due to this serotype. In our experience (9), infections caused by *H. influenzae* types e and f in Spain have not increased after vaccination campaigns; these pathogens produced mostly opportunistic infections in adults with underlying diseases.

The *H. influenzae* nontypeable isolation rate in England was 60% in the postvaccination era (34). In another study, 72.7% of invasive strains isolated after the start of vaccination were noncapsulated (17); these strains were also the most common *H. influenzae* invasive strains isolated in the present study (73.6%).

Nearly one in every four *H. influenzae* isolates was ampicillin resistant; all ampicillin-resistant isolates were β-lactamase producers. These rates are very similar to those recorded by The Spanish Surveillance Group for Respiratory Pathogens in 1,730 strains from respiratory tract isolates in Spain in 1998 and 1999 (27). In a collaborative European study (The Alexander Project), the overall prevalence of β-lactamase production was less than 12% in 1997 and 1998, although with marked geographical variation (32). In the present study, we have shown that capsulated strains were significantly more resistant to ampicillin, tetracycline, and chloramphenicol than were noncapsulated strains. This may explain why ampicillin resistance was more prevalent among children and isolates from CSF and blood. In a previous Spanish study, β-lactamase production was detected in 50% of Hib invasive isolates (12), a figure very similar to the 45.8% found in capsulated strains in the present study, even though only 62.5% of them were serotype b.

In the present study, a substantial prevalence of resistance to ampicillin, tetracycline, chloramphenicol, and trimethoprim-sulfamethoxazole was found in invasive *H. influenzae* against a background of decreased antibiotic consumption in the community. From 1996 to 2000, tetracycline and chloramphenicol consumption decreased from 0.70 to 0.58 and from 0.71 to 0.43 DDD/inhabitants day, respectively; amphenicol consumption was undetectable (Table 3). Decreases of 75 and 88% in tetracycline and cotrimoxazol consumption, respectively, have been reported in Spain from 1985 to 2000 (21). Antibiotic resistance can rapidly become more prevalent in a population, whereas its decline when antibiotic consumption is reduced is much slower (18). Furthermore, in areas with very high resistance rates, reduction in antibiotic pressure may have an even slower effect, especially where there is multidrug resistance (3, 24). There are various explanations for this phenomenon, including the possibility of resistance to different classes of antibiotics and coselection when only one of them is used (2, 18) and the reservoir of molecular mechanisms in species of the commensal flora and the exchange between these and pathogen species (1, 33).

Carriage of high-molecular-weight conjugative plasmids in Hib constitutes the genetic basis of resistance to ampicillin, chloramphenicol, and tetracycline (11, 25). Our results show a strong association between single and multiple resistances to these antibiotics and carriage of large conjugative plasmids (Table 1). However, the trimethoprim and clarithromycin resistance determinants are usually chromosomally mediated and are not associated with plasmid carriage (11). In the

present study, plasmids were not found in any of the 18 strains nonsusceptible to clarithromycin or the 21 strains with nonsusceptibility to cotrimoxazole alone. It is noteworthy that in our study, carriage of large conjugative plasmids, as detected by PCR, was common in resistant capsulated and noncapsulated *H. influenzae* strains.

In summary, we carried out a community study into the significance of invasive *H. influenzae* after widespread vaccination against *H. influenzae* serotype b. We collected data about the clinical diagnoses and the microbiological characteristics of the strains, including their molecular epidemiology, antibiotic resistance patterns, and antibiotic consumption in the community.

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